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3 **Comparative trophic impacts of two globally invasive cyprinid fishes reveal**
4 **species-specific invasion consequences for a threatened native fish**

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12 **Key words:** *Cyprinus carpio*; invasion; non-native species; stable isotope analysis;
13 isotopic niche.

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16 **Abstract**

17

18 1. Predicting the ecological consequences of invasions by non-native species is a
19 fundamental aspect of their risk-based management. As impacts can include the
20 negative consequences of resource sharing with native species, the application of
21 *in situ* cohabitation field experiments can test hypotheses relating to invasion
22 ecology via competitive interactions and processes. As fish are adaptable and
23 tractable experimental organisms, they are strong model species for use in
24 studies on competitive interactions.

25

26 2. The trophic consequences of invasion by two globally invasive freshwater fish,
27 common carp *Cyprinus carpio* and goldfish *Carassius auratus*, were tested on
28 the threatened native fish crucian carp *Carassius carassius*. Cohabitation
29 experiments, completed in pond enclosures, used all species in allopatric and
30 sympatric treatments using a substitutive design where the number of fish per
31 treatment was kept constant. Stable isotope analysis ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) assessed
32 alterations in the trophic ecology of each species across treatments, with growth
33 rates used to assess any consequent impacts on the fish.

34

35 3. When in sympatry with *C. auratus* and *C. carassius*, the *C. carpio* isotopic niche
36 was at a significantly lower trophic position compared to allopatry. This resulted
37 in niche overlap with *C. auratus*, whilst for *C. carassius*, their isotopic niche
38 shifted to a higher trophic position compared with allopatry. The growth rate of
39 *C. carpio* was always significantly higher in sympatry than in allopatry, whereas
40 growth rates for *C. carassius* and *C. auratus* were significantly depressed in *C.*
41 *carpio* presence. In contrast, the isotopic niche sizes and positions and growth

42 rates of the *Carassius* fishes were not significantly different between allopatry
43 and when they co-habited.

44

45 4. Plasticity in the isotopic niche of *C. carpio* resulted in significant alterations in
46 their trophic position between allopatry and sympatry and, when coupled with
47 their depressed growth in allopatry, suggests the competitive processes driving
48 this were intra-specific rather than inter-specific. This then resulted in
49 detrimental impacts in cohabiting *Carassius* fishes. These results emphasise that
50 ecological consequences of *C. carpio* in invaded freshwaters include impacts on
51 the trophic ecology of native fishes.

52

53

54 **Introduction**

55

56 Biological invasions are a pervasive agent of global change that can have substantial
57 consequences for native biota (Gozlan *et al.*, 2010; Simberloff *et al.*, 2013). Predicting
58 ecological impacts from invasive species is a cornerstone of their risk management,
59 enabling high impacting invaders to be identified (Copp *et al.*, 2009, 2016; Britton,
60 Gozlan & Copp, 2011). In aquatic ecosystems, recent developments include impact
61 predictions based on predator-prey interactions (Alexander *et al.*, 2014) and how these
62 influence population level impacts (Dick *et al.*, 2016). These predictive approaches do
63 not, however, assess competitive interactions between invasive and native species, thus
64 determining how invasive and native species interact when they share prey resources
65 also remains important for risk analysis (Tran *et al.*, 2015; Guo *et al.*, 2016).

66

67 Hypotheses that relate to how introduced and native species interact to determine the
68 outcome of introductions include ‘biotic resistance’, which suggests native species can
69 limit the probability of an introduced species from invading via consumptive and
70 competitive processes, and/ or through all available niches being occupied (Catford,
71 Jansson & Nilsson, 2009; Afos & Jackson, 2014). Conversely, the introduction of a
72 species with strong competitive abilities could displace a native competitor, facilitating
73 invasion and causing high ecological impacts (Catford, Jansson & Nilsson, 2009;
74 Gozlan *et al.*, 2010). Ecological theory suggests that when an introduced species
75 increases inter-specific competition for food resources within the community, larger
76 trophic niches can develop in competing species, as increasing dietary breadth should
77 reduce the intensity of the competitive interactions (Svanbäck & Bolnick, 2007; Bolnick
78 *et al.*, 2010). Alternatively, this might instead result in niches of competitors becoming
79 more specialised and divergent, as the competing species focus on feeding on prey

80 within their core niches (Van Valen, 1965; Jackson & Britton, 2014). Whilst these
81 alterations in trophic niches requires some plasticity in the diet of the species, both
82 responses are likely to facilitate the integration of the invader into the native community
83 and thus assisting colonisation and invasion (Tran *et al.*, 2015). Thus, these hypotheses
84 and ecological theory provide a framework for assessing how non-native species can
85 trophically integrate into a new environment following their introduction and their
86 subsequent impacts within the native community. As fish are adaptable and tractable
87 experimental organisms, they provide strong model systems for studies on competition,
88 including over relatively short periods of time (Ward, Webster & Hart, 2006).

89
90 Common carp *Cyprinus carpio* and goldfish *Carassius auratus* are globally significant
91 invasive fishes of the Cyprinidae family, with invasive populations of both species
92 present in Europe (Guo *et al.*, 2016). Whilst *C. carpio* is generally a benthic forager that
93 competes exploitatively, their diet can be highly plastic and incorporate prey items
94 taken throughout the water column (Garcia-Berthou, 2001; Britton *et al.*, 2007). The
95 reported ecological impacts of *C. carpio* on receiving ecosystems can be substantial and
96 generally relate to their ecological engineering activities (Parkos, Santuuci & Wahl,
97 2003; Koehn, 2004; Weber & Brown, 2009). Their ecological consequences for native
98 fishes that feed at similar trophic levels are less certain (Guo *et al.*, 2016), with it likely
99 that there are differences between impacts on native fish of 0-group *C. carpio* and adults
100 (Weber & Brown, 2017). Although current knowledge on *C. auratus* impacts has
101 mainly focused on genetic issues (e.g. Hänfling *et al.*, 2005; Wouters *et al.*, 2012), their
102 invasions have led to reported increases in turbidity and macrophyte damage (e.g.
103 Richardson, Whorisky & Roy, 1995). Whilst they are also omnivorous, their diet tends
104 to be more restricted to items in the benthos, including macro-invertebrates and detritus
105 (Lorenzoni *et al.*, 2007).

106 In contrast to *C. carpio* and *C. auratus*, the cyprinid crucian carp *Carassius carassius* is
107 native to ponds and river floodplains in north-west and central Europe (Tarkan *et al.*,
108 2016). Populations across their native range are considered threatened from issues
109 including habitat loss (Jeffries *et al.*, 2016; Tarkan *et al.*, 2016). The damaging
110 consequences for *C. carassius* of genetic introgression with *C. carpio* and *C. auratus*
111 are already recognised (Hänfling *et al.*, 2005; Wouters *et al.*, 2012). Conversely,
112 knowledge on the ecological impacts of *C. carassius* cohabitation with these invaders is
113 limited. A small number of field studies have suggested that when in sympatry with *C.*
114 *auratus*, the growth rates of *C. carassius* are similar to allopatry, suggesting minimal
115 impact (e.g. Tarkan *et al.*, 2009, 2010). By contrast, experimental evidence from tank
116 aquaria suggest that when food resources are limiting then both *C. carpio* and *C.*
117 *auratus* will out-compete *C. carassius* and result in their significantly depressed growth
118 rates (Busst & Britton, 2015). The combination of uncertainty in the invasion impacts
119 for *C. carassius* and their potential for sharing food resources with both invaders
120 suggests that these three species provide important and strong models for testing
121 hypotheses relating to the trophic interactions of native and invasive fishes.

122

123 Consequently, the aim of this study was to utilise an *in situ* cohabitation experiment,
124 based on stable isotope analysis (SIA), to determine how the trophic ecology of native
125 *C. carassius* was affected by invasive *C. carpio*, and how the invasion impacts of *C.*
126 *carpio* compared with another global invader, *C. auratus*. Given the apparent high diet
127 plasticity and global invasiveness of *C. carpio*, it was predicted that cohabitation with
128 *C. carpio* would result in significantly smaller trophic niche sizes of the *Carassius*
129 species, with consequential reductions in their growth rates, and thus indicating low
130 biotic resistance to *C. carpio* invasion.

131

132 **Materials and methods**

133

134 The experimental design was based on using allopatric and sympatric treatments in
135 which the total number of fish per replicate was maintained at a constant level ($n = 8$).

136 The allopatric treatments used each species individually, whilst the sympatric treatments
137 used two species per treatment ($n = 4$ fish per species), resulting in three sympatric
138 treatments: *C. carassius/ C. auratus*, *C. carassius/ C. carpio*, and *C. carpio/ C. auratus*.

139 Each treatment was replicated three times and used immature fish sourced from
140 aquaculture of starting length 55 - 70 mm (< 10 g in mass) (Table 1). As per Bašić and
141 Britton (2016) and Gutmann Roberts *et al.* (2017), the treatments were completed
142 within enclosures that sat within two adjacent ponds (nine enclosures per pond). The
143 larger ponds did not contain any fish and had been drained, emptied and refilled the
144 previous summer. The fish in the enclosures were thus the only fish present in the
145 ponds. The rationale for this was that the absence of fish outside of enclosures would
146 enable the unexploited component of the macro-invertebrate community to immigrate
147 into the enclosures, increasing the diversity of prey items available to the fishes and
148 ensuring the fish did not exhaust their prey resources.

149

150 Each enclosure comprised of an aluminium frame (length 1.05 m; width: 1.05 m;
151 height: 1.2 m) within a net (mesh: 7 mm) that prevented fish ingress and egress, but
152 allowed movements of water and invertebrates. The two ponds were approximately 400
153 m² in area and 1 m in depth, and were located on an aquaculture site in southern
154 England. Two ponds were utilised as high macrophyte growth resulted in some areas of
155 the individual ponds being unsuitable for the enclosures to be placed within them at the
156 start of the experiment. Thus, the enclosures in each pond were placed in areas where
157 macrophyte growth was still low, with at least 0.5 m between enclosures. Bird predation

158 was prevented via netting over the enclosures (15 mm mesh). The base of each
159 enclosure sat on the pond bed and settled into the silt, enabling fishes to access benthic
160 resources. During the experimental period, macrophytes grew through the mesh of all
161 enclosures, with areal cover by the conclusion of the experiment being between 40 and
162 50% in each enclosure (primarily *Elodea* spp.: Hydrocharitaceae). There were no
163 observed differences in macrophyte growth between enclosures and between
164 experimental treatments, suggesting the invasive fishes were of insufficient body size
165 and/ or abundance to influence macrophyte presence. The treatments were randomly
166 assigned to enclosures across the ponds.

167

168 The experiment commenced in May 2014 and lasted 100 days, with this duration based
169 on the rationale that according to the fish starting mass and the water temperatures
170 within the ponds, the fish dorsal muscle would undergo sufficient isotopic turnover to
171 represent their diet in the ponds (Thomas & Crowther, 2016). All fish were measured
172 (fork length) prior to their release into enclosures. Temperature loggers in the ponds
173 revealed mean temperatures of 18.3 ± 0.2 and $18.5 \pm 0.4^\circ\text{C}$ during the experiment. On
174 day 100, the fish were removed from the enclosures, euthanized (anaesthetic overdose,
175 MS-222) and taken to the laboratory where they were re-measured and a dorsal muscle
176 sample taken for SIA. At the same time, macro-invertebrate samples were taken from
177 the ponds using a sweep net within all major habitats. These samples enabled
178 comparison of community composition across the two ponds and provided samples for
179 SIA. *Gammarus pulex* (Gammaridae), Corixidae and Chironomid larvae dominated the
180 macro-invertebrate samples of both ponds; for SIA, triplicate samples were taken per
181 species and per pond, where one sample represented 3 to 6 individuals per species.
182 Whilst stable isotope samples of macrophytes and detritus were also taken ($n = 3$),
183 macro-invertebrates were considered as the primary food resources of the fishes as their

184 diets tend to primarily be based on macro-invertebrates at the length ranges used (Guo
185 *et al.*, 2016). All SIA samples were dried at 60 °C to constant mass before analysis
186 ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$) at the Cornell University Stable Isotope Laboratory, New York, USA,
187 where analytical precision associated with the $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ sample runs were
188 estimated at 0.42 and 0.15 ‰, respectively.

189

190 Comparison of the SI data of the macro-invertebrates between the two larger ponds
191 revealed considerable overlaps in their 95% confidence limits and meant that the SI data
192 of the fishes could be used and compared across both ponds without correction. The
193 trophic niche size of each fish species per treatments was calculated using the isotopic
194 niche, that, whilst related to the trophic niche, is acknowledged as also being influenced
195 by factors including growth rate and metabolism, and thus represents an approximation
196 of the trophic niche (Jackson *et al.*, 2011; Jackson *et al.*, 2012). It was calculated using
197 standard ellipse areas (SEA) in SIBER (Jackson *et al.*, 2011), a bivariate measure of the
198 distribution of individuals in isotopic space; as ellipses enclose \approx 40% of data, they
199 reveal the typical resource use of populations (Jackson *et al.*, 2012). Data per species
200 were combined across all three replicates per treatment to provide sample sizes
201 sufficient for calculation of standard ellipse areas ($n = 11$ to 18 ; Table 2). The minimum
202 number of individual fish used per species per replicate was three, ensuring that
203 individuals used in calculation of SEA were from across all replicates per treatment (*cf.*
204 Supplementary material for distribution of stable isotope data per replicate per treatment
205 and per pond). As the sample sizes were below 30 per species then a Bayesian estimate
206 of SEA (SEA_B) was used to test differences in niche sizes between species and
207 treatments, calculated using a Markov chain Monte Carlo simulation (10^4 iterations per
208 group) (Jackson *et al.*, 2011; Jackson *et al.*, 2012). Where SEA_B 95% confidence
209 intervals did not overlap between comparators, niche sizes were interpreted as

210 significantly different. To calculate isotopic niche overlap (%) between the species,
211 SEA_c was used, where the subscript 'c' indicates application of a small sample size
212 correction (Jackson *et al.*, 2012).

213

214 To test differences in SI data between species and treatments, $\delta^{15}N$ was converted to
215 trophic position (TP), where $TP_i = [(\delta^{15}N_i - \delta^{15}N_{base})/3.4] + 2$ (TP_i = trophic position of
216 the individual fish, $\delta^{15}N_i$ = fish isotopic ratio, $\delta^{15}N_{base}$ = mean macro-invertebrate
217 isotopic ratio, 3.4 = discrimination between trophic levels, and 2 = trophic position of
218 baseline macro-invertebrates) (Tran *et al.*, 2015). For each species, TP and $\delta^{13}C$ data
219 were tested for differences between treatments using linear mixed effects models
220 (LMEM). Due to the format of these data (i.e. treatments with replicates, with replicates
221 providing data from individual fish), using data from individual fish as replicates would
222 inflate the residual degrees of freedom and so models were fitted with pond enclosure as
223 a random effect to avoid pseudo-replication (Tran *et al.*, 2015). Differences between
224 species and treatments were then assessed using estimated marginal means and pairwise
225 comparisons (Bonferroni adjustments for multiple comparisons). The same model
226 structure tested differences in growth rate for each species per treatment, where the
227 growth rates of the fish species in each replicate per treatment was assessed using
228 incremental length (IL), calculated from $[(L_{t+1} - L_t)/t]/n$, where L_t = total initial length,
229 L_{t+1} = total final length, t = number of days, n = number of fish.

230

231 **Results**

232

233 Across the allopatric and sympatric treatments, the 95% confidence limits of the
234 isotopic niches (as SEA_B) of each species indicated that their niche sizes were not
235 significantly different between allopatry and sympatry (Fig. 1; Table 2). The LMEMs

236 testing the influence of species and treatment on TP and $\delta^{13}\text{C}$ were, however, generally
237 significant (Table 3). These models indicated that TP of *C. carassius* was significantly
238 lower in allopatry than when in sympatry with *C. carpio* ($P < 0.01$). This shift was,
239 however, not apparent when they were sympatric with *C. auratus* ($P = 0.53$; Table 3;
240 Fig. 1). The TP and $\delta^{13}\text{C}$ of *C. carpio* were both significantly higher in allopatry than
241 when in sympatry with either *C. carassius* or *C. auratus* (Table 3; Fig. 1). Thus, whilst
242 there were no significant differences in isotopic niche sizes across the treatments, there
243 were some significant differences in the positions of these niches in isotopic space
244 (Table 2, 3; Fig. 1).

245

246 Regarding the sharing of isotopic niches (as SEA_c), between species, the allopatric
247 treatments of *C. carassius* and *C. carpio* were discrete from each other in isotopic space
248 and this partitioning remained when the two species were in sympatry (Fig. 1). The
249 isotopic niches of *C. carassius* and *C. auratus* overlapped by 38% in allopatry, with this
250 overlap also apparent in sympatry where they shared 34% of their niches (Fig. 1). By
251 contrast, the isotopic niches of *C. auratus* and *C. carpio* did not overlap in allopatry, but
252 did in sympatry, where *C. carpio* shared 50% of *C. auratus* isotopic niche (Fig. 1).

253

254 The growth rates of the fishes, as incremental lengths, revealed that the influence of
255 treatment on growth rate was significant for each species (LMEM: $P < 0.01$ in all
256 cases). The growth rates of allopatric *C. carassius* and *C. auratus* were not significantly
257 different to when they were in sympatry ($P = 0.63$), despite the sharing of their isotopic
258 niches (Fig. 1, 2). However, the growth rates of both species were significantly reduced
259 when they were sympatric with *C. carpio* (*C. carassius*: $P < 0.01$; *C. auratus*: $P = 0.02$;
260 Table 1; Fig. 2). In contrast, the growth rates of *C. carpio* were significantly higher

261 when in sympatry with *C. carassius* ($P = 0.03$) and *C. auratus* ($P = 0.05$) when
262 compared with allopatry (Fig. 2).

263

264 **Discussion**

265

266 This *in situ* experiment assessed how the trophic ecology of native *C. carassius* was
267 affected by invasive *C. carpio*, and how the invasion impacts of *C. carpio* compared
268 with *C. auratus*, another globally invasive freshwater fish. Contrary to the prediction,
269 the trophic niche sizes of the *Carassius* fishes were not significantly different between
270 allopatry and sympatry with *C. carpio*. Conversely, it was the positions of these niches
271 in isotopic space that were affected; the *C. carpio* isotopic niche was at a lower trophic
272 position in sympatry with both *Carassius* fishes. This resulted in the displacement of the
273 *C. carassius* niche to a higher trophic position, perhaps through competitive exclusion.
274 These niche shifts impacted the growth of all species, with increasing growth rates for
275 *C. carpio* compared to allopatry but depressing those of both *Carassius* species. Thus,
276 as per the prediction, biotic resistance to *C. carpio* by *C. carassius* was negligible, with
277 the ecological impacts on *C. carassius* being considerably stronger from *C. carpio* than
278 *C. auratus*. Impacts of the invaders on macrophyte abundance were not detected in the
279 enclosures, perhaps due to their small body sizes at the start of the experiment. Thus,
280 the trophic impacts that are discussed subsequently focus on the intra- and inter-specific
281 interactions of the fishes, and their exploitation of prey communities.

282

283 *Cyprinus carpio* is already recognised as a globally invasive, high impact species
284 (Weber & Brown, 2009), being listed on the ‘World’s 100 worst invasive species’
285 (Lowe *et al.*, 2000). In the experiment, the re-organisation of the trophic structure by
286 invasive *C. carpio* across the different treatments impacted the ecology of both

287 *Carassius* species. This consequence complements other studies that have revealed
288 substantial ecological impacts by *C. carpio* on invaded freshwater ecosystems (Weber
289 & Brown, 2011). Indeed, *C. carpio* are recognised as a pest species in countries
290 including South Africa (Roberts *et al.*, 1995) and Australia (Koehn, 2004), where the
291 severity of their impacts is such that Koi Herpes Virus is likely to be released to assist
292 control and eradication efforts (McColl, Cooke & Sunarto, 2014; Di Giallonardo *et al.*,
293 2015). *Carassius* fishes and *C. carpio* are increasingly occurring in sympatry in invaded
294 regions (Britton *et al.*, 2010), with both invaders present in many European countries,
295 including Belgium (Verreycken *et al.*, 2007) and Spain (Elvira & Almodóvar, 2001).
296 Both *C. auratus* and *C. carpio* can have engineering impacts on invaded habitats,
297 including macrophyte loss (Richardson, Whorisky & Roy, 1995; Weber & Brown,
298 2009), thus additive or synergistic impacts could result in waters which they co-habit. In
299 our study, however, similar ecological impacts from *C. carpio* were recorded for *C.*
300 *auratus* and *C. carassius*, suggesting that the outcome of invasions would be dominance
301 by *C. carpio*, with the *Carassius* fishes being impacted as a result. It is anticipated that
302 this would primarily be via competitive interactions, at least in juvenile fishes, with this
303 aligned to Weber and Brown (2017) who suggested that the impacts of *C. carpio*
304 juveniles on native fish mainly occurs under food limitation. Adult *C. carpio*, a life-
305 stage not studied here, had more substantial impacts on native fish recruitment (Weber
306 & Brown, 2017), with Britton (2012) revealing that the mechanism of *C. carpio*
307 suppressing recruitment of other fishes was piscivory of young-of-the-year.

308

309 Despite this catalogue of ecological impacts of *C. carpio*, in much of Northwest Europe,
310 including in England and Wales, *C. carpio* is used to enhance recreational catch-and
311 release fisheries, with many fish stocked into angling ponds (Britton *et al.*, 2010). This
312 is despite predictions that climate change is likely to increase their ability to develop

313 invasive populations (Ruiz-Navarro, Gillingham & Britton, 2016). In contrast to *C.*
314 *carpio*, the experiment results suggested the ecological impacts of *C. auratus* on *C.*
315 *carassius* were low, with little indication that sharing of trophic space results in
316 depressed growth rates. However, the genetic impacts for native *C. carassius* from
317 introduced *C. auratus* can be substantial, with high genetic introgression via
318 hybridisation that threatens the status of native populations (Hänfling *et al.*, 2005;
319 Wouter *et al.*, 2012). In England at least, *C. auratus* is still used as a fish to supplement
320 the fishery performance of angling ponds, at least where their escape into the wider
321 environment is not possible (Britton *et al.*, 2010). The trophic similarity of the
322 *Carassius* fishes in the experiment also potentially suggests that *C. auratus* could
323 displace *C. carassius* in circumstances where resources are limiting, although this
324 remains speculative.

325

326 The plasticity in the isotopic niche of *C. carpio* between allopatry and sympatry
327 involved sympatric fish feeding at a lower trophic position but having faster growth rates.
328 The theoretical framework of the study focused on the consequences of inter-specific
329 competition for food resources between the fishes with testing of whether there were
330 alterations in niche size as competitive interactions with other species increased
331 (Svanbäck & Bolnick, 2007; Bolnick *et al.*, 2010). However, the advantage of using fish
332 as model species is that their indeterminate growth makes it possible to measure their
333 growth rates and correlate them with competitive success (Ward, Webster & Hart,
334 2006). Correspondingly, that the growth rates of *C. carpio* were elevated in sympatry
335 compared to allopatry suggests that the primary competitive process that resulted in the
336 reorganisation of the trophic structure of the experimental treatment was more likely to
337 be intra-specific than inter-specific. Thus, in the allopatric treatment where *C. carpio*
338 growth rates were depressed, the intra-specific interactions were sufficiently intense to

339 result in their exploitation of alternative food resources, shifting their isotopic niche to a
340 higher trophic level. A possible explanation is that when in allopatry, the *C. carpio* soon
341 exhausted their typical benthic food resources, forcing their shift to alternative
342 resources, such as those from prey items entering the enclosures from the larger ponds,
343 with these sufficient to result in the shift in their isotopic niche but insufficient to
344 support their growth rates as per the sympatric treatments. Given the less intense intra-
345 specific interactions in sympatry, then their preferred niche position was lower in the
346 food web and their growth rates were correspondingly higher. This then had the effect
347 of displacing the isotopic niche of co-habiting *C. carassius* to a higher trophic position,
348 with this sufficient to depress their growth rates compared to allopatry. This suggests
349 some complexity in the intra- and inter-specific interactions of these fishes, with further
350 experiments, including use of different densities and biomass per species per treatment,
351 required to fully understand the ecological processes driving these impacts.

352

353 A recent tank aquaria experiment revealed when food supply was limited, the
354 competitive interactions between *C. auratus* and *C. carassius* were asymmetric and
355 resulted in depressed *C. carassius* growth (Busst & Britton, 2015). Here, however, the
356 trophic and growth consequences for *C. carassius* from *C. auratus* were negligible,
357 despite their isotopic niches overlapping considerably and being contrary to ecological
358 theory on how niche sizes respond to resource sharing between natives and invaders
359 (e.g. Svanbäck & Bolnick, 2007; Bolnick *et al.*, 2010; Jackson & Britton, 2014). These
360 results suggest that although the fishes were occupying similar niches, their food
361 resources were not limiting or were at least sufficient to maintain their growth rates,
362 thus there was no requirement for either species to alter their niche sizes in sympatry
363 (Svanbäck & Bolnick, 2007; Bolnick *et al.*, 2010).

364

365 In entirety, these results of the experiment add to an increasing literature base over how
366 invasive fishes trophically integrate into native fish communities. For the Asian invader
367 *Pseudorasbora parva* (Cyprinidae), strong patterns of isotopic niche divergence were
368 apparent with native fishes in both allopatry and sympatry, with smaller niche sizes in
369 sympatry (Tran *et al.*, 2015). For the North American pumpkinseed *Lepomis gibbosus*
370 (Centrarchidae), reductions in isotopic niche size were demonstrated in three native
371 pond fishes in sympatry (Copp *et al.*, 2017). However, for riverine populations, strong
372 habitat partitioning with brown trout *Salmo trutta* (Salmonidae) resulted in minimal
373 trophic interactions between the two species (Jackson *et al.*, 2016). These studies thus
374 emphasise the strong species-specificity in the trophic interactions between invasive and
375 native fishes, and highlight that even where species appear functionally similar, this
376 does not necessarily serve as a strong predictor of the outcomes of resource sharing and
377 competitive interactions (Tran *et al.*, 2015; Copp *et al.*, 2017).

378

379 The results from these enclosure experiments contrast with the results of a previous
380 cohabitation aquaria experiment on the same fishes (*cf.* Busst & Britton, 2015). This is
381 important, given that patterns detected in ecological experiments completed under
382 controlled conditions do not always match those that manifest in more complex contexts
383 (Korsu, Huusko & Muotta, 2009; Spivak, Vanni & Mette, 2011). The relatively intense
384 competitive interactions of the fishes in the aquaria experiments of Busst and Britton
385 (2015) that resulted in depressed growth rates of *C. carassius* in the presence of both
386 invaders were only apparent in the pond enclosures in *C. carpio* presence. Given the
387 larger spatial scale, longer time period and availability of a range of natural food
388 resources, it is argued that the results from this pond enclosure experiment were more
389 likely to scale up and represent the impacts of the invaders on wild *C. carassius* than the
390 aquaria experiments. The enclosures were relatively small and thus there was still likely

391 to be some intensity in the interactions of the fishes, given their inability to utilise
392 alternative habitats. Therefore, different outcomes, such as strong patterns of niche
393 partitioning, could still develop between the fishes if larger experimental units were
394 used (e.g. larger enclosures or pond-scale experiments). Nevertheless, for this
395 experiment, it can be concluded that the ecological impacts of invasive *C. carpio* for
396 invaded freshwaters can include trophic impacts on threatened native species, adding to
397 the already substantial literature base on their harmful effects.

398

399 **References**

400

401 Alexander M.E., Dick J.T., Weyl O.L., Robinson T.B. & Richardson D.M. (2014)

402 Existing and emerging high impact invasive species are characterized by higher

403 functional responses than natives. *Biology Letters*, **10**, p.20130946.

404 Alofs K.M. & Jackson D.A. (2014) Meta- analysis suggests biotic resistance in

405 freshwater environments is driven by consumption rather than competition. *Ecology*,

406 **95**, 3259-3270.

407 Bašić T. & Britton J.R. (2016) Characterising the trophic niches of stocked and resident

408 cyprinid fishes: consistency in partitioning over time, space and body sizes. *Ecology*

409 *and Evolution*, **6**, 5093–5104.

410 Bolnick D.I., Ingram T., Stutz W.E., Snowberg L.K., Lau O.L. & Paull J.S. (2010)

411 Ecological release from interspecific competition leads to decoupled changes in

412 population and individual niche width. *Proceedings of the Royal Society of London:*

413 *Biological Sciences*, **277**, 1789-1797.

414 Britton J.R. (2012) Testing strength of biotic resistance against an introduced fish: inter-

415 specific competition or predation through facultative piscivory? *PloS one*, **7**,

416 p.e31707.

417 Britton J.R., Boar R.R., Grey J., Foster J., Lugonzo J. & Harper D.M. (2007) From
418 introduction to fishery dominance: the initial impacts of the invasive carp *Cyprinus*
419 *carpio* in Lake Naivasha, Kenya, 1999 to 2006. *Journal of Fish Biology*, **71**, 239-
420 257.

421 Britton J.R., Cucherousset J., Davies G.D., Godard M.J. & Copp G.H. (2010) Non-
422 native fishes and climate change: predicting species responses to warming
423 temperatures in a temperate region. *Freshwater Biology*, **55**, 1130-1141.

424 Britton J.R., Gozlan R.E. & Copp G.H. (2011) Managing non- native fish in the
425 environment. *Fish and Fisheries*, **12**, 256-274.

426 Busst G. & Britton J.R. (2015) Quantifying the growth consequences for crucian carp
427 *Carassius carassius* of competition from non-native fishes. *Ecology of Freshwater*
428 *Fish*, **24**, 489-492.

429 Catford J.A., Jansson R. & Nilsson C. (2009) Reducing redundancy in invasion ecology
430 by integrating hypotheses into a single theoretical framework. *Diversity and*
431 *Distributions*, **15**, 22-40.

432 Copp G.H., Vilizzi L., Mumford J., Fenwick G.V., Godard M.J. & Gozlan R.E. (2009)
433 Calibration of FISK, an invasiveness screening tool for non-native freshwater fishes.
434 *Risk Analysis*, **29**, 457-467.

435 Copp G.H., Russell I.C., Peeler E.J., Gherardi F., Tricarico E., Macleod A., Cowx I.G.,
436 Nunn A.D., Occhipinti-Ambrogi A., Savini D. & Mumford J. (2016) European Non-
437 native Species in Aquaculture Risk Analysis Scheme—a summary of assessment
438 protocols and decision support tools for use of alien species in aquaculture. *Fisheries*
439 *Management and Ecology*, **23**, 1–11.

440 Copp G.H., Britton J.R., Guo Z., Edmonds-Brown V.R., Pegg J., Vilizzi L. & Davison
441 P.I. (2017) Trophic consequences of non-native pumpkinseed *Lepomis gibbosus* for
442 native pond fishes. *Biological Invasions*, **19**, 25-41.

- 443 Di Giallonardo F. & Holmes E.C. (2015) Exploring Host–Pathogen Interactions through
444 Biological Control. *PLoS Pathogens*, 11, p.e1004865.
- 445 Dick J.T.A., Laverty C., Lennon J.J., Barrios-O'Neill D., Mensink P.J., Britton J.R.,
446 Médoc V., Boets P., Alexander M.E., Taylor N.G., Dunn A.M., Hatcher M.J.,
447 Rosewarne P.J., Crookes S., MacIsaac H.J., Xu M., Ricciardi A., Wasserman R.J.,
448 Ellender B.R., Weyl O.L.F., Lucy F.E., Banks P.B., Dodd J.A., MacNeil C., Penk
449 M.R., Aldridge D.C. & Caffrey J.M. (2017) Invader relative impact potential: a new
450 metric to understand and predict the ecological impacts of existing, emerging and
451 future invasive alien species. *Journal of Applied Ecology* DOI: 10.1111/1365-
452 2664.12849.
- 453 Elvira B. & Almodóvar A. (2001) Freshwater fish introductions in Spain: facts and
454 figures at the beginning of the 21st century. *Journal of Fish Biology*, **59**, 323–331.
- 455 García-Berthou E. (2001) Size-and depth-dependent variation in habitat and diet of the
456 common carp (*Cyprinus carpio*). *Aquatic Sciences*, **63**, 466-476.
- 457 Gozlan R.E., Britton J.R., Cowx I.G. & Copp G.H. (2010) Current knowledge on non-
458 native freshwater fish introductions. *Journal of Fish Biology*, **76**, 751-786
- 459 Guo Z., Sheath D., Amat-Trigo F. & Britton J.R. (2016) Comparative functional
460 responses of native and high impacting invasive fishes: impact predictions for native
461 prey populations. *Ecology of Freshwater Fish* DOI: 10.1111/eff.12297
- 462 Gutmann Roberts C., Bašić T., Amat Trigo F. & Britton J.R. (2017) Trophic
463 consequences for riverine cyprinid fishes of angler subsidies based on marine-
464 derived nutrients. *Freshwater Biology* DOI: 10.1111/fwb.12910.
- 465 Hänfling B., Bolton P., Harley M. & Carvalho G.R. (2005) A molecular approach to
466 detect hybridisation between crucian carp (*Carassius carassius*) and non-indigenous
467 carp species (*Carassius* spp. and *Cyprinus carpio*). *Freshwater Biology*, **50**, 403-417.

- 468 Jackson A.L., Inger R., Parnell A.C. & Bearhop S. (2011) Comparing isotopic niche
469 widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in
470 R. *Journal of Animal Ecology*, **80**, 595-602.
- 471 Jackson M.C., Donohue I., Jackson A.L., Britton J.R., Harper D.M. & Grey J. (2012)
472 Population-level metrics of trophic structure based on stable isotopes and their
473 application to invasion ecology. *PloS One*, **7**, e31757.
- 474 Jackson M.C. & Britton J.R. (2014) Divergence in the trophic niche of sympatric
475 freshwater invaders. *Biological Invasions*, **16**, 1095-1103.
- 476 Jackson M.C., Britton J.R., Cucherousset J., Guo Z., Stakėnas S., Gozlan R.E., Godard
477 M.G., Roussel J.M. & Copp G.H. (2016) Do non-native pumpkinseed *Lepomis*
478 *gibbosus* affect the growth, diet and trophic niche breadth of native brown trout
479 *Salmo trutta*? *Hydrobiologia*, **772**, 63-75.
- 480 Jeffries D.L., Copp G.H., Lawson Handley L., Olsén K.H., Sayer C.D. & Hänfling B.
481 (2016) Comparing RADseq and microsatellites to infer complex phylogeographic
482 patterns, an empirical perspective in the Crucian carp, *Carassius carassius*, L.
483 *Molecular Ecology*, **25**, 2997-3018.
- 484 Koehn J.D. (2004) Carp (*Cyprinus carpio*) as a powerful invader in Australian
485 waterways. *Freshwater Biology*, **49**, 882-894.
- 486 Korsu K., Huusko A. & Muotka T. (2009) Does the introduced brook trout (*Salvelinus*
487 *fontinalis*) affect growth of the native brown trout (*Salmo trutta*)?
488 *Naturwissenschaften*, **96**, 347-353.
- 489 Lorenzoni M., Corboli M., Ghetti L., Pedicillo G. & Carosi A. (2007) Growth and
490 reproduction of the goldfish *Carassius auratus*: a case study from Italy. In Gherardi F.
491 (ed.) *Biological invaders in inland waters: Profiles, distribution, and threats*.
492 Springer Netherlands. pp. 259-273.

493 Lowe S., Browne M., Boudjelas S. & De Poorter M. (2000) 100 of the world's worst
494 invasive alien species: a selection from the global invasive species database.
495 <http://s1.downloadmienphi.net/file/downloadfile2/169/1398476.pdf>. Last accessed
496 10/03/2016.

497 McColl K.A., Cooke B.D. & Sunarto A. (2014) Viral biocontrol of invasive vertebrates:
498 Lessons from the past applied to cyprinid herpesvirus-3 and carp (*Cyprinus carpio*)
499 control in Australia. *Biological Control*, **72**, 109-117.

500 Parkos III J.J., Santucci Jr. V.J. & Wahl D.H. (2003) Effects of adult common carp
501 (*Cyprinus carpio*) on multiple trophic levels in shallow mesocosms. *Canadian*
502 *Journal of Fisheries and Aquatic Sciences*, **60**, 182-192.

503 Richardson M. J., Whoriskey F. G. & Roy L. H. (1995) Turbidity generation and
504 biological impacts of an exotic fish *Carassius auratus*, introduced into shallow
505 seasonally anoxic ponds. *Journal of Fish Biology*, **47**, 576–58

506 Roberts J., Chick A., Oswald L. & Thompson P. (1995) Effect of carp, *Cyprinus carpio*
507 L., an exotic benthivorous fish, on aquatic plants and water quality in experimental
508 ponds. *Marine and Freshwater Research*, **46**, 1171-1180.

509 Ruiz-Navarro A., Gillingham P.K. & Britton J.R. (2016) Predicting shifts in the climate
510 space of freshwater fishes in Great Britain due to climate change. *Biological*
511 *Conservation*, **203**, 33-42.

512 Simberloff D. *et al.* (2013) Impacts of biological invasions: what's what and the way
513 forward. *Trends in Ecology and Evolution*, **28**, 58-66.

514 Spivak A.C., Vanni M.J. & Mette E.M. (2011) Moving on up: can results from simple
515 aquatic mesocosm experiments be applied across broad spatial scales?. *Freshwater*
516 *Biology*, **56**, 279-291.

- 517 Svanbäck R. & Bolnick D.I. (2007) Intraspecific competition drives increased resource
518 use diversity within a natural population. *Proceedings of the Royal Society of*
519 *London: Biological Sciences*, **274**, 839-844.
- 520 Tarkan A.S., Almeida D., Godard M.J., Gaygusuz Ö., Rylands M., Sayer C.D., Zięba G.
521 & Copp G.H. (2016) A review and meta- analysis of growth and life- history traits of
522 a declining European freshwater fish, crucian carp *Carassius carassius*. *Aquatic*
523 *Conservation: Marine and Freshwater Ecosystems*, **26**, 212-224.
- 524 Tarkan A.S., Almeida D., Godard M.J., Gaygusuz Ö., Rylands M., Sayer C.D., Zięba G.
525 & Copp G.H. (2016) A review and meta-analysis of growth and life-history traits of
526 a declining European freshwater fish, crucian carp *Carassius carassius*. *Aquatic*
527 *Conservation: Marine and Aquatic Ecosystems*, **26**, 212-224.
- 528 Tarkan A.S., Copp G.H., Zięba G., Godard M.J. & Cucherousset J. (2009) Growth and
529 reproduction of threatened native crucian carp *Carassius carassius* in small ponds of
530 Epping Forest, south- east England. *Aquatic Conservation: Marine and Freshwater*
531 *Ecosystems*, **19**, 797-805.
- 532 Tarkan A.S., Cucherousset J., Zięba G., Godard M.J. & Copp G.H. (2010) Growth and
533 reproduction of introduced goldfish *Carassius auratus* in small ponds of southeast
534 England with and without native crucian carp *Carassius carassius*. *Journal of*
535 *Applied Ichthyology*, **26**, 102-108.
- 536 Thomas S.M. & Crowther T.W. (2015) Predicting rates of isotopic turnover across the
537 animal kingdom: a synthesis of existing data. *Journal of Animal Ecology*, **84**, 861-
538 870.
- 539 Tran T.N.Q., Jackson M.C., Sheath D., Verreycken H. & Britton J.R. (2015) Patterns of
540 trophic niche divergence between invasive and native fishes in wild communities are
541 predictable from mesocosm studies. *Journal of Animal Ecology*, **84**, 1071-1080.

- 542 Van Valen L. (1965) Morphological variation and width of ecological niche. *American*
543 *Naturalist*, **99**, 377-390.
- 544 Ward A.J.W., Webster M.M. & Hart P.J.B. (2006) Intraspecific food competition in
545 fishes. *Fish and Fisheries*, **7**, 231–261.
- 546 Weber M.J. & Brown M.L. (2009) Effects of common carp on aquatic ecosystems 80
547 years after “carp as a dominant”: ecological insights for fisheries management.
548 *Reviews in Fisheries Science*, **17**, 524-537.
- 549 Weber M.J. & Brown M.L. (2017) Evaluating potential competitive bottlenecks
550 between invasive common carp and native bluegill and yellow perch. *Ecology of*
551 *Freshwater Fish* DOI: 10.1111/eff.12339.
- 552 Wouters J., Janson S., Lusková V. & Olsén K.H. (2012) Molecular identification of
553 hybrids of the invasive gibel carp *Carassius auratus gibelio* and crucian carp
554 *Carassius carassius* in Swedish waters. *Journal of Fish Biology*, **80**, 2595-2604.

Table 1. Mean initial and final fork lengths) of the fishes used in the pond enclosure experiment. Errors around the means represent standard errors.

Species	Context	Initial length			Final length			
		(mm)		<i>n</i>	(mm)		<i>n</i>	
<i>C. carassius</i>	Allopatric	61.5	± 1.3	24	77.0	± 1.7	24	
	Sympatric with	<i>C. auratus</i>	61.6	± 2.2	12	76.8	± 1.4	12
		<i>C. carpio</i>	61.4	± 1.5	12	67.7	± 1.3	11
<i>C. auratus</i>	Allopatric	64.9	± 1.4	24	87.0	± 1.4	22	
	Sympatric with	<i>C. carassius</i>	64.8	± 1.3	12	85.8	± 1.5	11
		<i>C. carpio</i>	63.9	± 2.0	12	78.1	± 1.5	10
<i>C. carpio</i>	Allopatric	64.1	± 1.4	24	74.5	± 1.5	24	
	Sympatric with	<i>C. carassius</i>	57.6	± 1.7	12	75.1	± 1.3	12
		<i>C. auratus</i>	59.9	± 2.0	12	75.2	± 2.6	12

Table 2. (A) Mean stable isotope data of the macro-invertebrate, macrophyte and detrital samples; (B) Mean carbon stable isotope and trophic position (TP), and trophic niche sizes (as standard ellipse areas; SEA_B with 95 % confidence intervals (CI) and SEA_c) of the fishes at the end of the pond enclosure experiment. Error around the means represent standard errors.

(A)

Taxa/ item	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)
Corixidae	-32.50 ± 0.43	3.83 ± 0.35
Chironomid larvae	-28.37 ± 0.92	6.12 ± 0.49
Gammaridae	-26.43 ± 0.82	5.18 ± 0.29
Odonata	-33.83 ± 0.65	3.98 ± 0.13
Detritus	-31.94 ± 0.49	3.71 ± 0.38
Macrophyte	-34.13 ± 0.42	2.53 ± 0.27

(B)

Species	Context	<i>n</i>	Mean $\delta^{13}\text{C}$ (‰)	Mean TP	SEA _B (‰ ²) (95 % CI range)	SEA _c (‰ ²)	
<i>C. carassius</i>	Allopatric	18	-25.76 ± 0.34	4.65 ± 0.05	1.39 (0.87 - 2.38)	1.35	
	Sympatric with	<i>C. auratus</i>	12	-25.36 ± 0.42	4.69 ± 0.06	1.62 (0.85 - 2.98)	1.49
		<i>C. carpio</i>	11	-24.51 ± 0.44	5.07 ± 0.06	1.18 (0.63 - 2.23)	1.43
<i>C. auratus</i>	Allopatric	18	-26.52 ± 0.34	4.46 ± 0.05	1.21 (0.78 - 2.00)	1.23	
	Sympatric with	<i>C. carassius</i>	11	-26.34 ± 0.44	4.48 ± 0.06	0.96 (0.55 - 1.91)	1.17
		<i>C. carpio</i>	10	-26.49 ± 0.46	4.68 ± 0.07	1.86 (0.92 - 3.64)	1.75
<i>C. carpio</i>	Allopatric	18	-26.28 ± 0.34	4.92 ± 0.05	3.31 (2.02 - 5.30)	3.45	
	Sympatric with	<i>C. carassius</i>	12	-28.09 ± 0.42	4.61 ± 0.06	2.77 (1.54 - 5.38)	3.03
		<i>C. auratus</i>	12	-28.06 ± 0.42	4.61 ± 0.06	2.77 (1.52 - 5.18)	3.15

Table 3. Outputs and significance of linear mixed effects models testing the difference in carbon stable isotope and trophic position per species across the pond enclosure experiment, where enclosure was the random effect on the intercept of each model. For each species, pairwise comparisons (with Bonferroni adjustment for multiple comparisons) tested the significance of the difference between the allopatric treatment and the sympatric treatments (*difference is significant at $P < 0.05$). For each species, model structure used replicate number as a random effect to avoid inflating the degrees of freedom that would otherwise occur had each fish been used as a replicate in the models.

$\delta^{13}\text{C}$				
Species in allopatry	AIC	P	Treatment	Difference
<i>Carassius carassius</i>	140.5	0.05	Sympatry with <i>Cyprinus carpio</i>	0.40
			Sympatry with <i>Carassius auratus</i>	1.25*
<i>Cyprinus carpio</i>	169.2	0.01	Sympatry with <i>Carassius carassius</i>	1.81*
			Sympatry with <i>Carassius auratus</i>	1.78*
<i>Carassius auratus</i>	121.5	0.91	Sympatry with <i>Carassius carassius</i>	0.18
			Sympatry with <i>Cyprinus carpio</i>	0.03
Trophic position				
Species in allopatry	AIC	P	Treatment	Difference
<i>Carassius carassius</i>	86.1	<0.01	Sympatry with <i>Cyprinus carpio</i>	0.41*
			Sympatry with <i>Carassius auratus</i>	0.03
<i>Cyprinus carpio</i>	93.5	<0.01	Sympatry with <i>Carassius carassius</i>	0.31*
			Sympatry with <i>Carassius auratus</i>	0.31*
<i>Carassius auratus</i>	76.3	0.86	Sympatry with <i>Carassius carassius</i>	0.02
			Sympatry with <i>Cyprinus carpio</i>	0.22

Figure captions

Figure 1. Stable isotope data and trophic niches (as standard ellipse area, SEA_e) of allopatric *C. carassius* and *C. auratus* (A); sympatric *C. carassius* and *C. auratus* (B); allopatric *C. carassius* and *C. carpio* (C); sympatric *C. carassius* and *C. carpio* (D); allopatric *C. carpio* and *C. auratus* (E) and sympatric *C. carpio* and *C. auratus* (F); ○ stable isotope data for *C. carassius*; ● stable isotope data for *C. auratus* and ▲ stable isotope data for *C. carpio*. Black solid lines mark the trophic niche of *C. carassius*; grey solid lines the trophic niche of *C. auratus* and black dashed lines the trophic niche of *C. carpio*. Note that for each species per treatment, individual stable isotope data were taken from fish in each replicate (minimum number per replicate = 3).

Figure 2. Growth rates, as mean incremental length, per species and treatment, where A: treatments involving *Carassius carassius* ('Cc'); B: *Carassius auratus* ('Ca'); and C: *Cyprinus carpio* ('Ccp'). *significantly different to allopatric ('allo') context ($P < 0.05$), as indicated by differences in estimated marginal means in the linear mixed effects model for each species, where enclosure was a random factor.

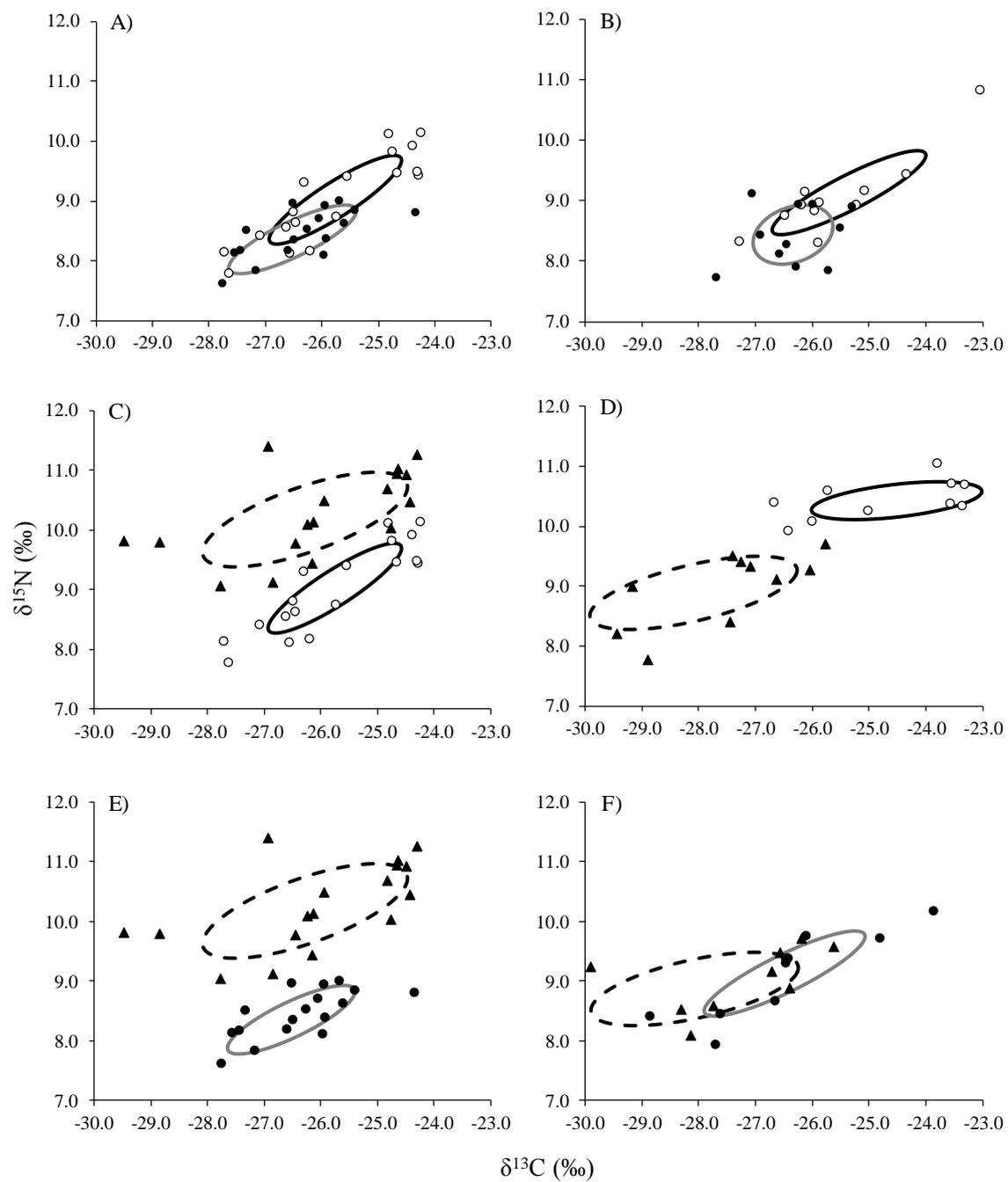


Figure 1.

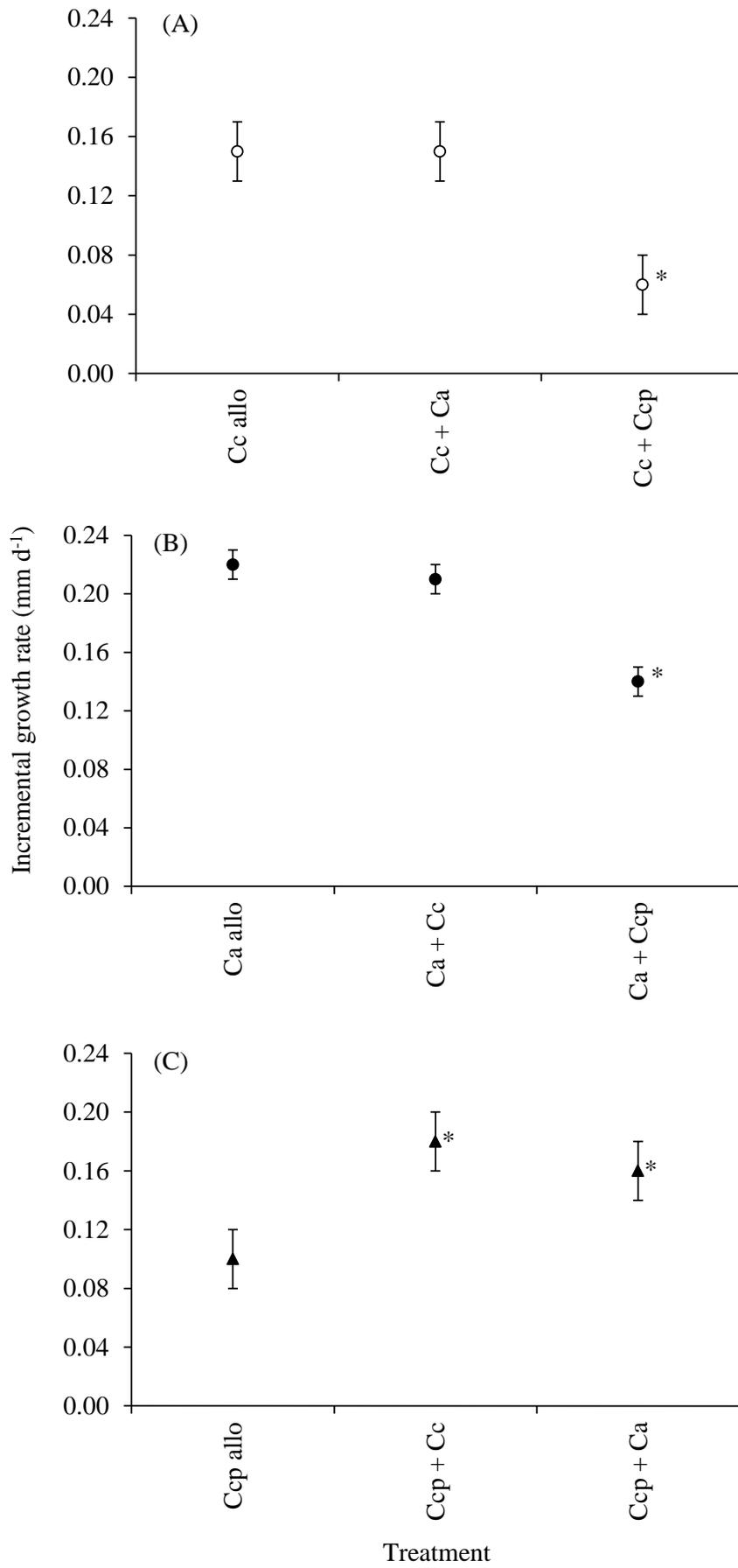


Figure 2.